PATENT
Docket No. 265.00240101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Stanton et al.	Group Art Unit:	1647
Serial No.:	09/641,802	Examiner:	R. Hayes
Confirmation No.:	5387		
Filed:	August 17, 2000		

For: USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF TO PROMOTE NEURONAL CELL DIFFERENTIATION

DECLARATION OF G. JOHN STANTON UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Dear Sir:

I, G. John Stanton, declare and say as follows:

1. I am a co-inventor of the subject matter claimed in the above-identified U.S. Patent Application Serial No. 09/641,802, filed August 17, 2000.

2. I received a Ph.D. from the University of Utah in 1967 and a B.A. from Stanford University, Palo Alto Ca in 1956.

   My research activities have included extensive work on cytokines and viruses focusing on immmuno-modulation and natural history of viruses. I have employed numerous human and other animal cell lines in this work, including observing the effects of cytokines and peptides on neural and leukocyte differentiation, . I have more than 140 publications

(abstracts, manuscripts, book chapters, and reviews) and have given numerous talks at symposia on immune-modulation and virus related topics.

3. I have read and am familiar with the Office Action mailed on September 10, 2002 with respect to the above identified application and make this Declaration in support of the patentability of the claims of patent application Serial No. 09/641,802.

4. On Page 5, item #12, of the Office Action, the Examiner asserts that the data provided within the application "discloses colostrinin-induced differentiation for only a single cell line, the PC12 cell line." Experiments performed in my laboratories in The Medical Research Building, Room 4.146, at the University of Texas Medical Branch, Galveston TX have now demonstrated the differentiation of a second cell line into neuron-like cells when contacted with colostrinin.

5. Our laboratory has observed the differentiation of SH-SY5Y cells into neuron-like cells when contacted with colostrinin. The SH-SY5Y cell line, derived from a human neuroblastoma, was obtained from the American Type Culture Collection and grown in HAM's F-10 medium containing 10% bovine calf serum supplemented with L-glutamine and 1 x antibiotic-antimycotic solution (100 units/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml Fungizone). Cultures were incubated at 37° C under 5% CO2/95% air. To evaluate the effect of colostrinin, SH-SY5Y cells were cultured with colostrinin "F", a newly prepared batch of colostrinin, was left on the cells for 30 minutes at 37° C and then dumped off and maintained on HAM's medium containing 2% FBS and antibiotics. Colostrinin "F" represents a new preparation of colostrinin used in this experiment but equivalent to other earlier preparations of colostrinin used to treat the PC-12 cell line as disclosed in the U.S. Patent Application Serial No. 09/641,802, filed August 17, 2000.

As a positive control, cells were treated with retinoic acid, a known agent that causes many neural cell lines to differentiate into neuronal-like cells . Retinoic acid was added to culture wells on Day 1 at 20 μM/well and left on the cells for the duration of the experiment. As a negative control, cells were mock treated for the duration of the experiment. Cultures were maintained under incubation in HAM's F-10 medium containing 10% bovine calf serum supplemented with L-glutamine and 1 x antibiotic-antimycotic solution (100 units/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml Fungizone). Cultures were incubated at 37° C under 5% CO2/95% air and observed under the microscope by three different individuals for morphometric changes and graded daily. The results are shown in Exhibit A.

6. Exhibit A shows colostrinin is capable of inducing neuronal cell differentiation in the SH-SY5Y cell line as compared to the negative and positive control groups.

7. I submit that one skilled in the art would conclude from the above data in Exhibit A that colostrinin is capable of inducing neuronal cell differentiation in multiple cell lines.

8. I further declare that statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: January 2, 2003

G. John Stanton

EXHIBIT A

Differentiation of SH-SY5Y Cells with Colostrinin

Treatment	Differentiation
Control *(Day 1)*[a]	– *(Day 5 & 7)*[b]
Retinoic Acid *(Day 1)*[a]	++++ *(Day 5)*[b]
Colostrinin "F" *(Day 5)*[a]	++++ *(Day 7)*[b]

++++ Approximately 100% of the cells
+ Approximately 25% of the cells
[a] Day of treatment
[b] Day cells observed